

BEST AVAILABLE COPY

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
22 December 2005 (22.12.2005)

PCT

(10) International Publication Number
WO 2005/121759 A2

(51) International Patent Classification⁷: G01N 27/00 (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(21) International Application Number: PCT/IB2005/002351

(22) International Filing Date: 24 May 2005 (24.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/574,036 24 May 2004 (24.05.2004) US
60/576,994 3 June 2004 (03.06.2004) US
60/577,034 3 June 2004 (03.06.2004) US

(71) Applicant (for all designated States except US): ALBATROS TECHNOLOGIES GMBH & CO. KG [DE/DE]; Lise-Meitner-Strasse 7, 48161 Münster (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BARTETZKO, Norbert [DE/DE]; Beversfördering 54, 59071 Hamm (DE). BARTETZKO, Robert [DE/DE]; Beversfördering 54, 59071 Hamm (DE). SPECHT, Bernfried [DE/DE]; Stadtörde 3, 48324 Sendenhorst (DE).

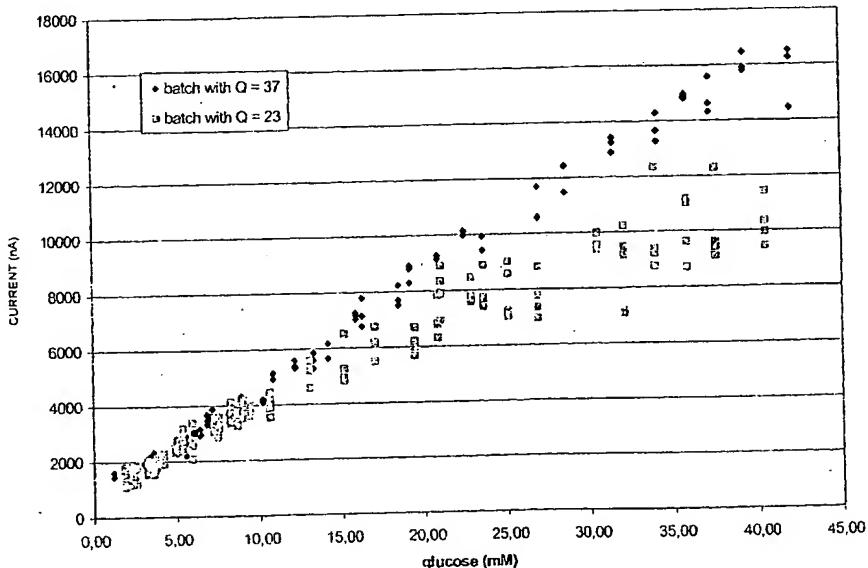
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: DEVICE AND METHOD FOR ANALYTE MEASUREMENT



WO 2005/121759 A2

(57) Abstract: A method is provided for analyte measurement using an analyte measuring device. The method comprises providing a substrate with at least one electrode; and covering at least the electrode with a layer containing zwitterionic compound and/or having the zwitterionic compound in a mediator layer to enable the analyte measuring device when coupled to a meter to generate a signal to noise ratio selected from one from of the following: better than 20, better than 50, better than 200, or better than 1000.

WO 2005/121759 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DEVICE AND METHOD FOR ANALYTE MEASUREMENT

5 BACKGROUND OF THE INVENTION

Technical Field:

The technical field relates analyte measurement and more specifically to sample filling and glucose sensing using novel disposable analyte testing devices and meter electronics for glucose spot monitoring.

10 Background Art:

Test strips are known in the medical health-care products industry for analyzing analyte levels such as but not limited to, glucose levels in blood. For this type of analysis, a drop of blood is typically obtained by making a small incision in the fingertip, creating a small wound, which generates a small blood droplet on the surface of the skin. A test strip is brought by the user to the blood droplet at the wound and engaged in a manner to bring blood to an analysis site on the test strip. The test strip is then coupled to a metering device which typically uses an electrochemical technique to determine the amount of glucose in the blood.

Early methods of using test strips required a relatively substantial volume of blood to obtain an accurate glucose measurement. This large blood requirement made the monitoring experience a painful one for the user since the user may need to lance deeper than comfortable to obtain sufficient blood generation. Alternatively, if insufficient blood is spontaneously generated, the user may need to "milk" the wound to squeeze enough blood to the skin surface. Neither method is desirable as they take additional user effort and may be painful. The discomfort and inconvenience associated with such lancing events may deter a user from testing their blood glucose levels in a rigorous manner sufficient to control their diabetes.

30 A further impediment to patient compliance is the amount of time that it takes for a glucose measurement to be completed. Known devices can take a substantial amount of time to arrive at a glucose level. The more time it takes to arrive at a measurement, the less the likely that the user will stay with their testing regime.

Known glucose test strips also use traditional manufacturing techniques that make it challenging to create the structures that may be used in improved, low volume analyte testing devices.

Accordingly, improved analyte measurement devices are desired to increase user
5 compliance and reduce the hurdles associated with analyte measurement.

SUMMARY OF THE INVENTION

The present invention provides solutions for at least some of the drawbacks discussed above. Specifically, some embodiments of the present invention provides

10 improved methods for sample fill and detection and is provided in one embodiment by screen-printing multiple layers and detection of sample arrival using a novel relay system. Other embodiments provide improved signal-to-noise ratio for measurement of analyte levels. At least some of these and other objectives described herein will be met by embodiments of the present invention.

15 In one embodiment, the present invention provides a method of forming an analyte testing device. The method comprises of providing a substrate; screen printing a plurality of electrode elements on top of the substrate; and screen printing at least one sample capture layer on top of the electrode elements to enable microfluidic capture of sample for analysis.

20 In another embodiment of the present invention, a method is provided for analyte measurement using an analyte measuring device. The method comprises providing a substrate with at least one electrode; and covering at least the electrode with a layer containing zwitterionic compound and/or having the zwitterionic compound in a mediator layer to enable the analyte measuring device when coupled to a meter to generate a signal
25 to noise ratio selected from one from of the following: better than 20, better than 50, better than 200, or better than 1000.

In another embodiment of the present invention, a method is provided for analyte measuring device. The method comprises providing a substrate with at least one electrode; and forming a hydrogel layer on the electrode, the hydrogel layer having a
30 thickness sufficient to enable the analyte measuring device when coupled to a meter to generate a signal to noise ratio selected from one from of the following: better than 20, better than 50, better than 200, or better than 1000.

In another embodiment of the present invention, a method is provided for analyte measurement. The method comprises providing a metering device having a counter relay; and providing an analyte testing device having a substrate, a plurality of electrodes on top of the substrate, and at least one sample capture layer on top of the electrode elements to enable microfluidic capture of sample for analysis; wetting two of the electrodes with sample fluid; measuring current the two electrodes until a threshold is reached; and closing the counter relay when the threshold is reached to allow amperometric measurement using three electrodes to occur.

In yet another embodiment of the present invention, a method is provided for analyte measurement. The method comprises providing a metering device having a counter relay in a closed starting condition; providing an analyte testing device having a substrate, a plurality of electrodes on top of the substrate, and at least one sample capture layer on top of the electrode elements to enable microfluidic capture of sample for analysis; wetting a working electrode with sample fluid causes the relay to move to an open condition; wetting a reference electrode causes measurement of current with the two electrodes until a threshold is reached; and closing the counter relay when the threshold is reached to allow amperometric measurement using three electrodes to occur.

In one embodiment of the present invention, the apparatus comprises a substrate; and a plurality of analyte detecting members on the substrate. The analyte detecting members arranged to form a plurality of concentric rings on the substrate. In another embodiment, the analyte detecting members include at least a working electrode and a reference electrode.

In yet another embodiment, the present invention relates to increasing printing density for manufacturing cost optimization by first printing the analyte detecting members on a sheet in high density then adhering them to the analyte detecting disk in a radial configuration such that there are several rings of analyte detecting members decreasing in density as one moves towards the center of the ring.

In one embodiment of the present invention, it should be understood that the variable amount of blood yield on lancing for shallow depth is between 0.2 and 0.5 μL . To optimize sample acquisition probability for samples of the order of 0.2 nL it would be useful to have a very low sensor volume, of the order of 0.1 μL . In one embodiment of the present invention, masking the 0.4 μL standard analyte detecting device used by

applicant and lowering the height of the cover can achieve 40 nL sample volume requirements without much optimization.

In one embodiment, the apparatus comprises a substrate; a plurality of conductive lines on the substrate; an insulating layer on the substrate; at least one working electrode and at least one counter electrode, each coupled to at least one conductive line; a cover film; and a support layer; a PSA layer, wherein the detecting member is masked to reduce the volume required for the detecting member is 40 nanoliter.

5 In one embodiment according to the present invention, a method is provided for forming an analyte testing device. The method comprises providing a substrate; screen printing a plurality of electrode elements on top of the substrate; and screen printing at least one sample capture layer on top of the electrode elements to enable microfluidic capture of sample for analysis. The method may include using a screen-printed sensor chamber. The method may include screen-printing a plurality of layers to form a sensor chamber. The method may include covering the electrode elements with a hydrophilic 10 layer to enable signal to noise ratio selected from one from of the following: better than 20, better than 50, better than 200, or better than 1000. The electrode elements may be formed by printing five layers of material. The sample elements may be formed by printing two layers of material. The method may include covering a mediator layer on the electrode elements with a layer containing zwitterionic compound and/or having the 15 zwitterionic compound in a mediator layer to enable a signal to noise ratio selected from one from of the following: better than 20, better than 50, better than 200, or better than 1000.

20

25 In another embodiment of the present invention, a method is provided for analyte measurement using an analyte measuring device. The method comprises providing a substrate with at least one electrode; covering at least the electrode with a layer containing zwitterionic compound and/or having the zwitterionic compound in a mediator layer to enable the analyte measuring device when coupled to a meter to generate a signal to noise ratio selected from one from of the following: better than 20, better than 50, better than 200, or better than 1000. The layer may be about 4 microns thick. The layer 30 may be less than 30 microns thick.

In one embodiment according to the present invention, a method is provided for analyte measurement using an analyte measuring device. The method comprises

providing a substrate with at least one electrode; and forming a hydrogel layer on the electrode, wherein the hydrogel layer having a thickness sufficient to enable the analyte measuring device when coupled to a meter to generate a signal-to-noise ratio selected from one from of the following: better than 20, better than 50, better than 200, or better than 1000. The hydrogel layer may be about 4 microns thick. The hydrogel layer may be less than 30 microns thick.

In one embodiment according to the present invention, a method is provided for analyte measurement. The method comprises providing a metering device having a counter relay; providing an analyte testing device having a substrate, a plurality of electrodes on top of the substrate, and at least one sample capture layer on top of the electrode elements to enable microfluidic capture of sample for analysis; wetting two of the electrodes with sample fluid; measuring current the two electrodes until a threshold is reached; and switch over the counter relay when the threshold is reached to allow amperometric measurement using three electrodes to occur.

In yet another embodiment of the present invention, a method is provided for analyte measurement. The method comprises providing a metering device having a counter relay in a closed starting condition; providing an analyte testing device having a substrate, a plurality of electrodes on top of the substrate, and at least one sample capture layer on top of the electrode elements to enable microfluidic capture of sample for analysis; starting the measurement by switching the relay between the two electrode system and the three electrode system to detect the direction of the sample flow; wetting a working electrode with sample fluid causes a current flow; and switching the counter relay over and wait until the threshold is reached to allow amperometric measurement using three electrodes to occur.

In yet another embodiment of the present invention, a method is provided for fluid sample analyte measurement. The method comprises providing a metering device having a counter relay; providing an analyte testing device having a three electrode system; wetting two of the electrodes with the fluid sample to determine sample fill; and beginning analyte sample measurement when all three electrodes are in contact with the fluid sample. Optionally, the analyte sample measurement does not occur until all three electrodes are in contact with the fluid sample.

In yet another embodiment of the present invention, a method is provided for fluid sample analyte measurement. The method includes providing a metering device having a switch; providing an analyte testing device having a three electrode system; wherein the switch has a first configuration for electrically coupling a first electrode and a second electrode on the testing device, wherein the switch has at least a second configuration for coupling a second electrode and a third electrode on the testing device; and monitoring fluid flow through the testing device based on when fluid sample contacts each electrode.

5 The monitoring may include generating a signal when the first electrode and second electrode are both in contact with fluid. The monitoring may include generating a signal

10 when the second electrode and third electrode are both in contact with fluid. The monitoring may include moving the switch into the second configuration when fluid covers a portion of the first electrode and fluid has begun to contact the second electrode.

15 The switch may be a counter relay that sends signal to a microcontroller.

In yet another embodiment of the present invention, a device is provided for

15 analyte measurement. The device may include a substrate; and a plurality of analyte detecting members on the substrate, wherein the analyte detecting members are arranged to form a plurality of concentric rings on the substrate. The analyte detecting members may include at least a working electrode and a reference electrode. The analyte detecting members may include at least a working electrode and a reference electrode and a

20 hydrogel membrane. The substrate may include at least 100 analyte detecting members. The substrate may include at least 75 analyte detecting members. The substrate may include at least 50 analyte detecting members. The device may include a cartridge containing a plurality of penetrating members, wherein the cartridge is coupled to the substrate. The device may include a cartridge containing a plurality of penetrating

25 members, the cartridge coupled to the substrate so that the analyte detecting members are positioned along an outside rim of the cartridge.

In yet another embodiment of the present invention, a device is provided for

analyte measurement. The device includes a substrate; and a plurality of analyte

30 detecting members on the substrate, wherein the analyte detecting members are arranged to form a geometric pattern on the substrate. The substrate may include at least 50 analyte detecting members. The geometric pattern may be selected from one of: lines, linear arrays, circular arrays, square arrays, polygonal arrays, or triangular arrays. The device

may include a cartridge containing a plurality of penetrating members, the cartridge coupled to the substrate so that the analyte detecting members are positioned along an outside rim of the cartridge. The device may include a sample capture assembly that includes a film mesh at the front end to draw blood to the analyte detecting members, and 5 the film can be advanced after use to ensure fresh piece for each lancing event.

In yet another embodiment of the present invention, an analyte detecting member is provided that includes a substrate; a plurality of conductive lines on the substrate; an insulating layer on the substrate; at least one working electrode and at least one counter electrode, each coupled to at least one conductive line; a cover film; a support layer; a 10 PSA layer. There may be a hole in cover film and a hole in PVC support. There may be a hole in PSA layer; wherein the detecting member is masked to reduce the volume required for the detecting member is 40 nanoliters or less. In other embodiments, the volume is 100 nanoliters or less. In one embodiment, the diameter of the hole in cover film: is 1 mm, diameter of the hole in PVC support: 1.6 mm, and diameter of the hole in 15 PSA layer: 2.6 mm. The device may use a radial cartridge having a plurality of the analyte testing members. A ring may be provided having a plurality of the analyte testing members.

In yet another embodiment of the present invention, a kit is provided comprising a device as described in the paragraph above; a lancing device, wherein the device of the 20 above paragraph is mounted on the lancing device; instructions for use instructing the user to apply no or minimal pressure against the device during lancing and applying pressure post lancing for spontaneous blood generation.

In yet another embodiment of the present invention, a method is provided for reducing volume used in an analyte testing device. The method comprises masking a 25 surface of an 0.4 μL analyte detecting member; and lowering the height of the cover to achieve 40 nL sample volume requirements: In one embodiment, the method comprises masking a surface of an analyte detecting member; and lowering the height of the cover to achieve lower sample volume requirements. The method comprises masking a surface of a greater than 0.2 μL analyte detecting member; and lowering the height of the cover 30 to achieve 100 nL sample volume requirements. The method may include providing a radial cartridge and forming a plurality of analyte detecting members on the cartridge. The method may include providing a ring and forming a plurality of analyte detecting

members on the ring. The method may include providing a ring and forming a plurality of analyte detecting members on the ring. The method may include coupling the ring to a cartridge containing a plurality of penetrating members.

In yet another embodiment of the present invention, a method is provided for

5 manufacturing. The method may include drilling of holes into PVC-support; printing of the conductive lines; printing of the insulating layer; printing of reference and counter electrodes; printing of the working electrode (wherein in one embodiment the composition is: 50% mediator / 100% buffer compounds / 50% GOD), printing of the hydrophilic membrane (wherein in one embodiment the composition is: PAA/CHAPS); printing of

10 the spacer layer. In process-control, there may be measurement of background and saturation current. The method may include printing of the PSA-layer; applying of mesh (for the mesh structure); applying of the cover film 126_2 having drilled holes; and stamping process. The printing of the working electrode may use a composition: 50% mediator / 100% buffer compounds / 50% GOD. The in process-control may include

15 measurement of background and saturation current after printing spacer layer. The method may include applying of the cover film having drilled holes. The method may include control of the resistance when printing the conductive lines.

A further understanding of the nature and advantages of the invention will become apparent by reference to the remaining portions of the specification and drawings.

20

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Figure 1 shows a graph of signal to noise ratio.

Figures 2-4 are charts comparing signal to noise ratios for various embodiments.

Figures 5A and 5B show embodiments of a testing device according to the present

25 invention.

Figure 6 is a cross-sectional view of one embodiment of the present invention.

Figures 7A and 7B are schematics of electronic circuits according to the present invention.

Figures 8A-8B show one embodiment of the present invention using as switcher.

30 Figures 9A-9C show one embodiment of the present invention using as switcher.

Figure 10 shows one embodiment of a disc for use with the present invention.

Figure 11 is an exploded perspective view of one embodiment according to the present invention.

Figure 12 shows a ring according to the present invention.

Figure 13 shows a cross-sectional view of one embodiment of the present invention.

Figure 14 shows several embodiments according to the present invention.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. It may be noted that, as used in the specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a material" may include mixtures of materials, reference to "a chamber" may include multiple chambers, and the like. References cited herein are hereby incorporated by reference in their entirety, except to the extent that they conflict with teachings explicitly set forth in this specification.

In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

"Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not. For example, if a device optionally contains a feature for analyzing a blood sample, this means that the analysis feature may or may not be present, and, thus, the description includes structures wherein a device possesses the analysis feature and structures wherein the analysis feature is not present.

Embodiments of the present invention provide a variety improvements over the known art. For example, the present invention provides a low volume analyte detecting member, optimized signal to background noise designs, improved methods for manufacturing analyte detecting members, a method of connecting a hydrophilic layer to a conductive layer, and a method for sensing sample arrival.

1) In one embodiment, the present invention provides optimized signal to background noise designs. Current ratio of signal to background optimization may use

one of the following to obtain improved results: 1) use of a hydrogel with zwitterionic compounds or 2) changes in hydrophilic layer dimensions to obtain improved signaling. Currently available test strips have a maximum signal to noise ratio of about 20.

Experimental data for analyte testing devices with and without hydrophilic layer and

5 with/without zwitterionic compound shows that the present invention may achieve significantly improved signal to noise ratio. In other embodiments, the mediator content, geometrical design of the electrode and the sample chamber also influence the signal. Some embodiments may achieve signal to noise ratio greater than 20. Some embodiments may achieve signal to noise ratio greater than 25. Some embodiments may achieve signal
10 to noise ratio greater than 30. Some embodiments may achieve signal to noise ratio greater than 35.

In one embodiment, because the reduced form of the mediator is embedded into the reaction zone of one embodiment of the present analyte testing device, a current can be observed after applying the voltage. This generates the oxidized form of the mediator,

15 which is able to react with the reduced form of the enzyme glucose-oxidase. For this particular example, in the absence of glucose (using phosphate buffered saline only (pH 7.4)) a (background) current, will be detected. As a nonlimiting example, adding 500 mM glucose in buffer and applying a voltage, the saturation current can be determined. The excess glucose is so that the device is not limited by lack of glucose when making the
20 saturation current measurement. The original background current may be caused by the oxidation of mediator by the electrode. In one embodiment of the present invention, the mediator exists in the reduced form in the carbon paste, and is active in the oxidized form during the measurement. There is typically always a little reduced mediator present, which gives the background signal (which is termed the "glucose independent" current).
25 Approaches to reduce this background current in the art are to try and oxidize the mediator before the measurement. Most are inefficient at achieving this and so there is a lot of reduced mediator available leading to a larger background current and hence lower Q (in the range of 20). Chronoamperometric methods look at last section of the graph so that a low amount of reduced mediator is observed, as most of the mediator has
30 disappeared from the electrode paste and does not contribute to background. Coulometric methods look at charge generated during the entire reaction. At the start, there is a lot of background signal, and at the end there is little, but the result is the integration over the

whole time, resulting in total charge and hence the sum of background signal as a result.. It is therefore inherently difficult with the coulometric methods to get a high Q.

The ratio of the saturation current to the background current (Q) (saturation current / background current) correlates with the measuring range of any test strips.

- 5 Constructing an analyte testing device with the highest ratio possible so that the measurement range is extended to upwards of 40 mM glucose. A Q of 20 is generally the maximum ratio observed in the art, and is the result of lack of hydrophilic membranes and the surfactant composed of zwitterions (zwitterions are both a negatively and positively charged).
- 10 Experimental data for analyte testing devices with and without hydrophilic layer and with/without zwitterionic compound shows that the present invention may achieve significantly improved results. In one embodiment, the combination of hydrogels, CHAPS, and then the dimensional change to the hydrogel layer may be used to improve signaling.

- 15 As mentioned previously, thickness of the hydrogel can also improve signal to noise ratio. It should be understood that chamber dimension and layer thickness might influence the signal. Several micrometers in change to they hydrogel layer may be sufficient to alter the ratio. In one embodiment, the thickness of the layer is about 4 micrometers. Increasing the thickness also increases the ratio. Unfortunately, increased thickness of the layer may also slow the diffusion rate of the analyte. The slope is the diffusion dependent current, and if a thick hydrophilic member is used, the diffusion rate of glucose to electrodes is decreased. Number of glucose molecules per second is slower. In some embodiments, the thickness will not exceed 30 micrometers. In other embodiments, the thickness will not exceed 50 micrometers
- 20

- 25 Referring now to Figure 1, the importance of a high Q value is reflected in the ability to measure glucose at the higher concentrations. Figure 1 shows the results of glucose concentration versus current for two different batches of test strips, which have components known to generate different values of Q. As seen, the test strip batch having a ratio of 23 has only a measuring range up to 20 mM glucose in whole blood, whereas a
- 30 batch having a ratio of 37 shows a measuring range up to about 40 mM glucose in whole blood. Both batches have same background. (In one embodiment of the present invention, the background current is about 1000 nA). The graph of Figure 1 confirms the same

12

precision in the low range of glucose, but higher precision in the high range as well as a broader range of glucose concentration measurement at the high glucose levels. At low glucose concentration (2.5 mM) the ratio between the slope and the background is important and the precision is important. Q can be increased by applying (e.g. screen-printing) a hydrophilic membrane over the working electrode.

Figure 2 shows that application of a hydrophilic membrane to the analyte testing device construction increases Q. The membrane effectively functions by enhancing the concentration of the mediator at the surface of the working electrode. The net result is that the actual concentration of the mediator in the reaction zone is higher in comparison to the batch having no hydrophilic membrane.

Referring now to Figure 3, Q can be increased even further by optimizing the formulation of the paste for the hydrophilic such as using of a detergent as part of the hydrophilic membrane. The magnitude of the increase of course depends on the type of detergent used. The Q value for trips containing Triton or CHAPS are compared in Figure 15. Using the zwitter-ionic detergent CHAPS in the hydrophilic membrane Q was increased by a factor of 1.5 in comparison to the non-ionic detergent Triton X-100.

Referring now to Figure 4, further modifications of analyte testing devices according to the present invention lead to ratios having values up to 148 (see Figure 4), though the detergent tends to be rather unstable. Different types of analyte testing device 20 constructions have given rise to different values of Q in Figure 4. Batch 1 is an analyte testing device without membrane; similar to what is available in the art the resultant Q is 23. Batch 2 is an analyte testing device constructed with the proprietary hydrophilic membrane, increasing to Q to 37. Batch 3 and 4 contain the hydrophilic membrane and different zwitterionic detergent compounds, resulting in almost double of the value for Q. 25 Batch 5 is the highest operating Q value of about 150, but the detergent has proven to be rather unstable. In one embodiment, Q of the present invention is in the range of 60-80.

2) In another embodiment, the present invention provides a method for manufacturing an analyte testing device using screen printing a plurality of layers, such as but not limited to seven layers wherein adhesive is counted as one layer. Currently 30 available glucose test strips have sample chambers created by laminating step. In one embodiment, the sample capture structures of the present invention may be formed by laying the following layers.

1. Conductive layer
2. Insulating layer
3. Reference and counter electrode
4. Working electrode
5. Hydrophilic membrane
6. Spacer layer
7. Adhesive layer

Figure 5A shows dimension of the sample capturing structure without mesh (GS-SC 1, in its original form). The structure, in some other embodiments, may include a mesh. By way of example and limitation, a channel 40 may have a width of 0.5 mm. The opening over each electrode is also about 0.5mm. The contact pads may have a size of 1.2mm. An opening may have a diameter of 1.0mm.

As seen in Figure 5B, one embodiment of the present invention comprises of six layers which may be manufactured by seven printing steps. In this method, five steps construct the electrode elements 30 while 2 steps account for the two layers 32 and 34 that comprise the microfilling features. The present invention comprises printing channels that are used for hydrophilic filling. $30 \mu\text{m}^2$ printing is challenging but being done. In one embodiment, a $30 \mu\text{m}^2 \times 50 \mu\text{m}$ thick electrode would use 1nL volume. Embodiments of the present invention may only require a sample volume of $0.6 \mu\text{L}$.

In one embodiment, the noise floor issues mean that the lowest amperage for dilution may 10nA. In one embodiment, $0.4 \mu\text{L} \times 5\text{mm}^2 \times 80 \mu\text{m}$ area X thickness. In one embodiment, $0.2 \mu\text{L} \times 4\text{mm}^2 \times 50 \mu\text{m} = 200\text{nA}$ signal. In one embodiment, $1\text{mm}^2 \times 100 \mu\text{m} = 0.1 \mu\text{L}$ is challenging but doable for a 10nA signal, the present invention could go 1/20 of the $0.2 \mu\text{L}$. In one embodiment, the present invention could theoretically do $0.1 \text{mm}^2 \times 0.05 \mu\text{L}$ or $0.005 \mu\text{L}$ 0.5nL .

Referring now to Figure 6, one embodiment is shown connecting hydrophilic layer to the conductive layer. The "mushroom" shaped electrode may connect the conductive layer to the hydrophilic membrane with increased surface area using a drop through geometry. The mushroom-shaped cap of the electrode is configured to increase the surface area in contact with the layer above.

Figure 6 shows a cross-section of the analyte detecting members. In this embodiment, a substrate 100 is provided. On top of this substrate, a carbon paste is

provided to form conducting layers 102 for a screen-printed three-electrode system. A spacer layer 104 may also be provided. The reference and the counter electrodes 142 and 143 may be made of a formulation of Ag/AgCl. The analyte detecting member may be based on chrono-amperometry measurement technique using glucose oxidase (Gox) 5 enzyme and N,N,N',N'-Tetramethyl-p-phenylenediamine (TMPD), as electron transfer mediator. Although not limited to the following, the working electrode 140 may optionally comprise of carbon paste blended with Gox, the mediator, a buffer and a thinner. A hydrophilic layer or membrane 108 is provided on top of the electrodes. In some embodiments, only the working electrode 140 has the hydrophilic layer 108. It 10 should be understood that the hydrogel may be formed in a variety of shapes including but not limited to rectangular, square, polygonal, circular, triangular, any single or multiple combination of shapes, or the like.

Embodiments of the present invention may use a three-electrode system. For testing devices with "bad" mediators, there is normally a dummy electrode, which is used 15 to reduce background current. The reference electrode of the present invention is a more advanced design as it conveys the potential. It is independent of sample variability, so it achieves a constant potential between the working electrode and the counter electrode.

Referring now to Figures 7 and 8, another embodiment of the present invention provides a meter relay 200 for sensing sample arrival and monitoring flow of sample in 20 the testing device. In one embodiment of the present invention, Figure 7A shows a system with electronics that do not handle switching and coupled to a testing device 150. Figure 7B shows a system with electronics that two of the electrodes may be used for an auto-trigger function while all three electrodes may be used for the measurement. Figure 7B shows an analyte testing device 150 coupled to a switch 200. In this embodiment, the 25 switch is located in the analog portion of the meter electronics. Other embodiments may locate the switch in a digital part of the circuit. A switch, relay or other type of general switch device may be used to switch monitoring of various electrodes. The switch 200 allows for monitoring of the first and second electrodes which contact the fluid sample. The switch 200 when moved into a second configuration allows for monitoring of the 30 second and third electrodes in the testing device that contact the fluid sample. The signal is transferred to the digital portion 203 of the circuit which includes a microcontroller 205 for processing information from the various sets of electrodes.

As seen in the embodiment of Figure 8A, in the start condition, the counter relay 200 is open as indicated by arrow 208. A reference electrode lead 210, counter electrode lead 212, and working electrode 214 lead is shown. As seen in Figure 4, if the blood sample wets the reference electrode as indicated by arrow 216, the measurement starts in 5 the two electrode-system modes until the current reaches the threshold level. Then in Figure 8B, the counter relays 200 closes as indicated by arrow 220 and the amperometric measurement for the detection of the glucose occurs in the three electrode-system mode. Threshold level (value for auto trigger) is adjustable from 50 to 2,000 Na. Thus, a two 10 electrode-system used for auto-trigger function, three electrode-system used for the measurement. In summary, blood sample wets the reference electrode, the counter relays is switched, and the amperometric measurement starts. It should also be understood that instead of measurement using the first two electrodes, they can also be used to monitor fluid flow in the capillary. For example, when the blood covers the first electrode and contacts the second electrode, the signal begins to flow. The relay 200 then switches to 15 monitor the time it takes for blood (after it contacts the second electrode, typically the working electrode) until the blood reaches the third electrode. The time it takes for the blood to flow from working electrode to the third electrode is monitored to know the flow velocity in the capillary. If the time it takes for blood to flow from the second to third electrode exceeds a threshold, then the measurement may be discarded. The threshold 20 may be one minute, two minutes, or more.

Referring now to Figure 9A, another embodiment of the present invention will now be described. As indicated by arrow 230, in the starting condition, the counter array 200 is closed. If the blood sample B wets the working electrode as indicated by arrow 232 as seen in Figure 9B, a current will be detected (non-regulated potential). After that, 25 the relays are switched to the open modus as indicated by arrow 234. Thus, when blood sample wets the working electrode, the counter relays is switched to the open modus. If the blood sample wets the reference electrode, the measurement starts in the two electrode-system mode until the current reaches the threshold level. Then, the counter relays closes as indicated by arrow 236 and the amperometric measurement for the 30 detection of the glucose occurs in the three electrode-system mode. This feature gives the opportunity to measure the flow rate of the blood. This feature also gives the opportunity to reload the system with more blood. However, if the time difference (current peak, if

working electrode has been wet, and current peak, if reference electrode has been wet) is too long; the measurement is omitted (time control for reloading). Threshold level (value for auto trigger) is adjustable from 50 to 2,000 nA. The threshold level is a compromise between possible non-fluid caused currents and the slope. Although blood is the example used herein for illustrative purposes, it should be understood that other body fluids may be used, depending on the analyte being measured. Thus, when blood samples wets the reference electrode, the counter relays is switched to the closed modus, the amperometric measurement starts.

It should be understood that the present invention not only detects that the capillary is filled when blood reaches the third electrode, but it also monitors the movement of blood to and through the electrochemical cell or testing device. Detecting movement into and through the channel may have its benefits. The present invention monitors the movement of the sample. In the present invention, the potential is detected between two electrodes. There is a current flow when two electrodes are contacted by blood and a signal is generated. The potential is then between the two different electrodes, which allows current flow and a signal. The second signal shows that the capillary is filled.

As discussed above, the present invention may use a switcher. A general switcher may be used to monitor two different locations. Monitoring in the same sense that they see the entry of the blood, when the capillary is filled, and thus the velocity of fluid flow. This is based on the time. There are some electrochemical that starts when touch working electrode and when capillary is filled. The partial covering of working and filling the whole channel are two aspects that the present invention monitors. The allows the monitoring of blood through the sample device. Maximum time that is allowed to fill the whole channel is also determined.

Referring now to Figure 10, a still further embodiment of a cartridge according to the present invention will be described. Figure 10 shows one embodiment of a cartridge 300 which may be removably inserted into an apparatus for driving penetrating members to pierce skin or tissue. The cartridge 300 has a plurality of penetrating members 302 that may be individually or otherwise selectively actuated so that the penetrating members 302 may extend outward from the cartridge, as indicated by arrow 304, to penetrate tissue. In the present embodiment, the cartridge 300 may be based on a flat disc with a number of

penetrating members such as, but in no way limited to, (25, 50, 75, 100, ...) arranged radially on the disc or cartridge 800. It should be understood that although the cartridge 300 is shown as a disc or a disc-shaped housing, other shapes or configurations of the cartridge may also work without departing from the spirit of the present invention of placing a plurality of penetrating members to be engaged, singly or in some combination, by a penetrating member driver.

Each penetrating member 302 may be contained in a cavity 306 in the cartridge 300 with the penetrating member's sharpened end facing radially outward and may be in the same plane as that of the cartridge. The cavity 306 may be molded, pressed, forged, or otherwise formed in the cartridge. Although not limited in this manner, the ends of the cavities 306 may be divided into individual fingers (such as one for each cavity) on the outer periphery of the disc. The particular shape of each cavity 306 may be designed to suit the size or shape of the penetrating member therein or the amount of space desired for placement of the analyte detecting members 808. For example and not limitation, the cavity 306 may have a V-shaped cross-section, a U-shaped cross-section, C-shaped cross-section, a multi-level cross section or the other cross-sections. The opening 810 through which a penetrating member 302 may exit to penetrate tissue may also have a variety of shapes, such as but not limited to, a circular opening, a square or rectangular opening, a U-shaped opening, a narrow opening that only allows the penetrating member to pass, an opening with more clearance on the sides, a slit or the other shapes.

In this embodiment, after actuation, the penetrating member 302 is returned into the cartridge and may be held within the cartridge 300 in a manner so that it is not able to be used again. By way of example and not limitation, a used penetrating member may be returned into the cartridge and held by the launcher in position until the next lancing event. At the time of the next lancing, the launcher may disengage the used penetrating member with the cartridge 300 turned or indexed to the next clean penetrating member such that the cavity holding the used penetrating member is position so that it is not accessible to the user (i.e. turn away from a penetrating member exit opening). In some embodiments, the tip of a used penetrating member may be driven into a protective stop that hold the penetrating member in place after use. The cartridge 300 is replaceable with a new cartridge 300 once all the penetrating members have been used or at such other time or condition as deemed desirable by the user.

Referring still to the embodiment in Figure 10, the cartridge 300 may provide sterile environments for penetrating members via seals, foils, covers, polymeric, or similar materials used to seal the cavities and provide enclosed areas for the penetrating members to rest in. In the present embodiment, a foil or seal layer 320 is applied to one 5 surface of the cartridge 300. The seal layer 320 may be made of a variety of materials such as a metallic foil or other seal materials and may be of a tensile strength and other quality that may provide a sealed, sterile environment until the seal layer 320 is penetrate by a suitable or penetrating device providing a preselected or selected amount of force to open the sealed, sterile environment. Each cavity 306 may be individually sealed with a 10 layer 320 in a manner such that the opening of one cavity does not interfere with the sterility in an adjacent or other cavity in the cartridge 800. As seen in the embodiment of Figure 10, the seal layer 320 may be a planar material that is adhered to a top surface of the cartridge 800.

Depending on the orientation of the cartridge 300 in the penetrating member 15 driver apparatus, the seal layer 320 may be on the top surface, side surface, bottom surface, or other positioned surface. For ease of illustration and discussion of the embodiment of Figure 10, the layer 320 is placed on a top surface of the cartridge 800. The cavities 306 holding the penetrating members 302 are sealed on by the foil layer 320 and thus create the sterile environments for the penetrating members. The foil layer 320 20 may seal a plurality of cavities 306 or only a select number of cavities as desired.

In a still further feature of Figure 10, the cartridge 300 may optionally include a plurality of analyte detecting members 308 on a substrate 822 which may be attached to a bottom surface of the cartridge 300. The substrate may be made of a material such as, but not limited to, a polymer, a foil, or other material suitable for attaching to a cartridge and 25 holding the analyte detecting members 308. As seen in Figure 7, the substrate 322 may hold a plurality of analyte detecting members, such as but not limited to, about 10-50, 50-100, or other combinations of analyte detecting members. This facilitates the assembly and integration of analyte detecting members 308 with cartridge 300. These analyte detecting members 308 may enable an integrated body fluid sampling system where the 30 penetrating members 302 create a wound tract in a target tissue, which expresses body fluid that flows into the cartridge for analyte detection by at least one of the analyte detecting members 308. The substrate 322 may contain any number of analyte detecting

members 308 suitable for detecting analytes in cartridge having a plurality of cavities 306. In one embodiment, many analyte detecting members 308 may be printed onto a single substrate 322 which is then adhered to the cartridge to facilitate manufacturing and simplify assembly. The analyte detecting members 308 may be electrochemical in nature.

5 The analyte detecting members 308 may further contain enzymes, dyes, or other detectors which react when exposed to the desired analyte. Additionally, the analyte detecting members 308 may comprise of clear optical windows that allow light to pass into the body fluid for analyte analysis. The number, location, and type of analyte detecting member 308 may be varied as desired, based in part on the design of the cartridge,

10 number of analytes to be measured, the need for analyte detecting member calibration, and the sensitivity of the analyte detecting members. If the cartridge 300 uses an analyte detecting member arrangement where the analyte detecting members are on a substrate attached to the bottom of the cartridge, there may be through holes (as shown in Figure 76), wicking elements, capillary tube or other devices on the cartridge 300 to allow body

15 fluid to flow from the cartridge to the analyte detecting members 308 for analysis. In other configurations, the analyte detecting members 308 may be printed, formed, or otherwise located directly in the cavities housing the penetrating members 302 or areas on the cartridge surface that receive blood after lancing.

The use of the seal layer 320 and substrate or analyte detecting member layer 822 20 may facilitate the manufacture of these cartridges 10. For example, a single seal layer 320 may be adhered, attached, or otherwise coupled to the cartridge 300 as indicated by arrows 324 to seal many of the cavities 306 at one time. A sheet 322 of analyte detecting members may also be adhered, attached, or otherwise coupled to the cartridge 300 as indicated by arrows 325 to provide many analyte detecting members on the cartridge at 25 one time. During manufacturing of one embodiment of the present invention, the cartridge 300 may be loaded with penetrating members 302, sealed with layer 320 and a temporary layer (not shown) on the bottom where substrate 322 would later go, to provide a sealed environment for the penetrating members. This assembly with the temporary bottom layer is then taken to be sterilized. After sterilization, the assembly is taken to a 30 clean room (or it may already be in a clear room or equivalent environment) where the temporary bottom layer is removed and the substrate 322 with analyte detecting members is coupled to the cartridge as shown in Figure 10. This process allows for the sterile

assembly of the cartridge with the penetrating members 302 using processes and/or temperatures that may degrade the accuracy or functionality of the analyte detecting members on substrate 322. As a nonlimiting example, the entire cartridge 300 may then be placed in a further sealed container such as a pouch, bag, plastic molded container, 5 etc...to facilitate contact, improve ruggedness, and/or allow for easier handling.

In some embodiments, more than one seal layer 320 may be used to seal the cavities 306. As examples of some embodiments, multiple layers may be placed over each cavity 306, half or some selected portion of the cavities may be sealed with one layer with the other half or selected portion of the cavities sealed with another sheet or 10 layer, different shaped cavities may use different seal layer, or the like. The seal layer 320 may have different physical properties, such as those covering the penetrating members 302 near the end of the cartridge may have a different color such as red to indicate to the user (if visually inspectable) that the user is down to say 10, 5, or other number of penetrating members before the cartridge should be changed out.

15 Referring now to Figure 11, one embodiment of an analyte detecting device according to the present invention will now be described. In one embodiment, they may be printed in sheets, then placed at high density in concentric rings or other configurations (such as but not limited to lines, linear arrays, circular arrays, square arrays, polygonal arrays, triangular arrays, or other patterns) on the disk. In Figure 11, this embodiment of 20 device 400 is shown in an exploded view. It includes a hydrophilic cover 402, a spacer 404, a hydrophilic member 406, a working electrode 408, a counter and reference electrodes 410, an insulating layer 412, conductive lines 414, and a substrate 416.

Referring now to Figure 12, one embodiment of the present invention involves producing the analyte testing members 420 in the arrays on sheets at high density. In one 25 embodiment, it is possible to die cut these high density manufactured analyte testing members and place them on the ring 450, though increase the density by adhering the analyte testing members in consecutive rings diminishing in size as the diameter decreases going from the outside of the ring to the inside. This may decreases manufacturing cost 12 fold in comparison to GSD. Printed area for GSR in 150 sq mm 30 and for GSD 1875 sq mm.

Microfluidics is proven technology there is no additional requirement for microfluidics for sample transfer from the finger to a remote analyte testing member since

this is already taken care of by sip-in fluid acquisition. However, some thought into transferring sample to the inner rings is desired, as these will be removed from the disposable front end by a longer fill channel. Some embodiments may use a larger priming volume.

5 Embodiments of the present invention may also reduce the blood volume to 100 nL. None required to prime the microfluidic sample capture channel (for the outside rings). For the inside rings, a slightly longer sample fill channel may be required.

10 Some embodiments may include a seal on the analyte testing members in a separate manner. Desiccant may also be printed in the paste forming the microfluidic channel and therefore we may not need any extra packaging. Inner analyte testing members may include sample priming volume the closer they are to the center of the disc.

15 Referring now to Figure 13, yet another embodiment of the present invention will now be described. In this embodiment, the invention may comprise of mounting an analyte testing disc 580 so that the analyte testing members 590 are on the outside rim of the cartridge 600 housing at least one penetrating member 602. In some embodiments, a hydrophilic cover member 604 may be included. In this embodiment, sample capture can be achieved by using a film mesh 610 at the front end to draw blood to the analyte testing members 590, and the film 610 can be advanced after use to ensure fresh piece each time. The advantages of this embodiment are that the cartridge 600 and analyte testing member disk 580 remain the same, the contamination problem is addressed, there is analyte testing member-analyte testing member-isolation because of hydrophilic cover film, as well as analyte testing member-lancet isolation. The contact to the meter is easily achieved, in one embodiment, through pins to establish electrical contact between conductive lines coupled to members 590 and the meter (not shown).

20 25 In the present embodiment, the stability of analyte testing members 590 is not problematic. There is a solution for producing the air vent, at the beginning of the measurement; a needle can be used to pierce a hole into the hydrophilic cover film at the end of the capillary. A 2-point alignment may be used with the present invention. The lancet exit point is through the through mesh 'center'. The system may include a new indexing mechanism to move the disc in addition to the cartridge 600. This dual rotating or indexing device may be used. In one embodiment, this may allow for denser numbers of analyte testing members (i.e. one analyte detecting disc may be used for every two

penetrating member cartridges for instance). Desiccant may be a desiccant-coated Al film. In addition, precise film transportation may also be used.

Referring now to Figures 5A and 14, another embodiment of the present invention will now be described. It should be understood that the analyte detecting members

5 described herein may be used with a cartridge such as that in Figure 10 or any other cartridge discussed herein. This embodiment provides a method for further lowering the minimum fluid sample volume used for a fluid device.

In the present embodiment of the invention, to control the volume of blood or fluid sample, the height of the channel was decreased so that the volume was dropped to

10 get $0.2\mu\text{L}$ from a starting sample volume of $0.6\mu\text{L}$. From a production point of view the opening of the channel with the dye cutting sometimes the channel got crimped. This can be overcome with another cutting process, only a limitation when producing the prototype.

In one embodiment, the height of the channel was about $50\ \mu\text{m}$. In another

15 embodiment, the design (3.0) has a shortened channel, and an increased height to $80\ \mu\text{m}$. This reduces any issues with high Haematocrit or high viscosity so that it was difficult to get the blood into the channel translating into no reading. There is only a 1% inconsistency of fill with the $80\ \mu\text{m}$. Some data has been acquired on this analyte testing device. The volume of the sample chamber is $0.24\ \mu\text{L}$ by calculation; there may be a
20 variation, though they have shown that a variation in the height of the sensor does not affect the performance. To increase the efficiency of the system in terms of sample capture and measurement, the thought was to move to a lower volume requiring sensor configuration. The present invention improves volume without reducing the height of the channel because of the constraints with respect to the blood, and without shortening the
25 channel..

In the $0.4\ \mu\text{L}$ (4.3 design), to achieve lower volumes, a strip is to a $0.1\ \mu\text{L}$ volume. In this embodiment, the width of the channel is reduce to $500\ \mu\text{m}$ by masking. Channel was $1000\ \mu\text{m}$. In this embodiment, there is alignment of the 4 layers above the electrode. The present invention may fill in the gaps between the electrodes. The ratio of the height
30 the electrodes and the dimension of the gap. In the configuration where you have a fat ($2000\ \mu\text{m}$) working electrode and a fat ($500\ \mu\text{m}$ gap) the situation for printing the layers is less stringent. The fat electrode configuration is in the 4.3 strip. For the working

electrode in the GSSC design, the working electrode is 500 µm and the gap is thinner (250µm). This is challenging because there may be undulations in the wall. This is now a 0.1 µL proposition.

As seen in Figure 5A, the size of the sample capturing structure in its original form is 6.15 mm x 5 mm (without mesh, see figure 5A) as well as 7.75 mm x 5 mm (with mesh).

Figure 14 shows an overview of the three different variants of sample capturing structures (enlarged structure) Owing to the better handling as well as a using of a half-automatic stamping procedure the size of both structures were enlarged to 7.2 mm x 40 mm (see Figure 13). Various embodiments shown in Figure 13 are described below.

10 Variation 1: GS-SC 1 without mesh, both holes have a diameter of 1 mm

Variation 2: GS-SC 2 with mesh 620,

diameter of the hole in cover film: 1 mm

diameter of the hole in PVC support: 1.6 mm

diameter of the hole in PSA layer: 2.6 mm

15 Variation 3: GS-SC 3 without mesh,

diameter of the hole in cover film: 1 mm

diameter of the hole in PVC support: 1.6 mm

diameter of the hole in PSA layer: 2.6 mm

20

One embodiment of a method for manufacturing these elements are described herein. Manufacturing of sample capturing structures (batch size: 10 sheets) may include:

I) drilling of holes into PVC-support

II) printing of the conductive lines: control of the resistance

25 III) printing of the insulating layer

IV) printing of reference and counter electrodes

V) printing of the working electrode

(Composition: 50% mediator / 100% buffer compounds / 50% GOD)

VI) printing of the hydrophilic membrane

(Composition: PAA/CHAPS)

30 VII) printing of the spacer layer

In process-control: measurement of background and saturation current

- VIII) printing of the PSA-layer
- IX) applying of mesh (for the mesh structure)
- X) applying of the cover film 126_2 having drilled holes
- XI) stamping process

5 Electrochemical characterization of sample capturing structures may include:

- I) Determination of background and saturation current ($n = 24 - 48$)

Measurements were done during the maturation process (up to 3-4 weeks), directly after the maturation process, after the maturation process over a long period

- II) Determination of KM as well as determination of the slope within the linear range 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 50, 100, 200, 300, 400, 500 mM glucosein buffer

10 20 different glucose solutions, measurement per concentration: $n = 8$

- III) Measurement of 40 different blood samples covering a glucose range from 1 to 40 mM glucose, measurement per concentration: $n = 8$

15 Evaluation: Error-Grid-Analysis

- a) applying of the samples with a pipetman
- b) applying of the samples with finger

20 While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. For example, with any of the above embodiments, the hydrogel layer may have a shape such as but not limited to circular, square, rectangular, triangular, hexagonal, polygonal, oval, or single or multiple combinations of any of the above. The layer containing the 25 zwitterionic material (which in some embodiments is the same as the hydrogel) may also have these shapes. In some embodiments, there may be more than one layer of any of the hydrogel layer or layer with zwitterionic material. It should be understood that the electrode may be formed in a variety of shapes including but not limited to rectangular, square, polygonal, circular, triangular, any single or multiple combination of shapes, or 30 the like.

The publications discussed or cited herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an

admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed. All publications mentioned herein are incorporated herein by reference to disclose and describe the structures and/or methods in connection with which the publications are cited. U.S. 5 Provisional Applications Ser. Nos. 60,574,036 (Attorney Docket No. 38187-2730), 60/576,994 (Attorney Docket No. 38187-2734), and 60/577,034 (Attorney Docket No. 38187-2736) are fully incorporated herein by reference for all purposes.

10 Expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended, therefore, that the invention be defined by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

WHAT IS CLAIMED IS:

- 1 1. A method of forming an analyte testing device, the method
2 comprising:
 - 3 providing a substrate;
 - 4 screen printing a plurality of electrode elements on top of said substrate;
 - 5 and
 - 6 screen printing at least one sample capture layer on top of said electrode
7 elements to enable microfluidic capture of sample for analysis.
- 1 2. The method of claim 1 further comprising using a screen-printed
2 sensor chamber.
- 1 3. The method of claim 1 further comprising screen-printing a
2 plurality of layers to form a sensor chamber.
- 1 4. The method of claim 1 further comprising covering the electrode
2 elements with a hydrophilic layer to enable signal to noise ratio selected from one from of
3 the following: better than 20, better than 50, better than 200, or better than 1000.
- 1 5. The method of claim 1 wherein said electrode elements are formed
2 by printing five layers of material.
- 1 6. The method of claim 1 wherein said sample elements are formed
2 by printing two layers of material.
- 1 7. The method of claim 1 further comprising covering a mediator
2 layer on the electrode elements with a layer containing zwitterionic compound and/or
3 having the zwitterionic compound in a mediator layer to enable a signal to noise ratio
4 selected from one from of the following: better than 20, better than 50, better than 200, or
5 better than 1000.
- 1 8. A method of analyte measurement using an analyte measuring
2 device, said method comprising:
 - 3 providing a substrate with at least one electrode;

4 covering at least the electrode with a layer containing zwitterionic
5 compound and/or having the zwitterionic compound in a mediator layer to enable the
6 analyte measuring device when coupled to a meter to generate a signal to noise ratio
7 selected from one from of the following: better than 20, better than 50, better than 200, or
8 better than 1000.

1 9. The method of claim 8 wherein said layer is about 4 microns thick.

1 10. The method of claim 8 wherein said layer is less than 30 microns
2 thick.

1 11. A method of analyte measurement using an analyte measuring
2 device, said method comprising:
3 providing a substrate with at least one electrode; and
4 forming a hydrogel layer on said electrode, said hydrogel layer having a
5 thickness sufficient to enable the analyte measuring device when coupled to a meter to
6 generate a signal-to-noise ratio selected from one from of the following: better than 20,
7 better than 50, better than 200, or better than 1000.

1 12. The method of claim 11 wherein said hydrogel layer is about 4
2 microns thick.

1 13. The method of claim 11 wherein said hydrogel layer is less than 30
2 microns thick.

1 14. A method of analyte measurement comprising:
2 providing a metering device having a counter relay;
3 providing an analyte testing device having a substrate, a plurality of
4 electrodes on top of said substrate, and at least one sample capture layer on top of said
5 electrode elements to enable microfluidic capture of sample for analysis;
6 wetting two of said electrodes with sample fluid;
7 measuring current the two electrodes until a threshold is reached; and
8 switch over the counter relay when said threshold is reached to allow
9 amperometric measurement using three electrodes to occur.

1 15. A method of analyte measurement comprising:

2 providing a metering device having a counter relay in a closed starting
3 condition; .
4 providing an analyte testing device having a substrate, a plurality of
5 electrodes on top of said substrate, and at least one sample capture layer on top of said
6 electrode elements to enable microfluidic capture of sample for analysis;
7 starting the measurement by switching the said relay between the two
8 electrode system and the three electrode system to detect the direction of the sample flow;
9 wetting a working electrode with sample fluid causes a current flow; and
10 switching the counter relay over and wait until said threshold is reached to
11 allow amperometric measurement using three electrodes to occur.

1 16. A method of fluid sample analyte measurement comprising:
2 providing a metering device having a counter relay;
3 providing an analyte testing device having a three electrode system;
4 wetting two of said electrodes with said fluid sample to determine sample
5 fill; and
6 beginning analyte sample measurement when all three electrodes are in
7 contact with said fluid sample.

1 17. The method as in claim 16 wherein analyte sample measurement
2 does not occur until all three electrodes are in contact with said fluid sample.

1 18. A method of fluid sample analyte measurement comprising:
2 providing a metering device having a switch;
3 providing an analyte testing device having a three electrode system;
4 wherein the switch has a first configuration for electrically coupling a first
5 electrode and a second electrode on the testing device, wherein the switch has at least a
6 second configuration for coupling a second electrode and a third electrode on the testing
7 device;
8 monitoring fluid flow through the testing device based on when fluid
9 sample contacts each electrode.

1 19. The method as in claim 18 wherein the monitoring comprises
2 generating a signal when the first electrode and second electrode are both in contact with
3 fluid.

1 20. The method as in claim 18 wherein the monitoring comprises
2 generating a signal when the second electrode and third electrode are both in contact with
3 fluid.

1 21. The method as in claim 18 wherein the monitoring comprises
2 moving the switch into the second configuration when fluid covers a portion of the first
3 electrode and fluid has begun to contact the second electrode.

1 22. The method as in claim 18 wherein the switch is a counter relay
2 that sends signal to a microcontroller.

1 23. A device for analyte measurement comprising: [2734]
2 a substrate; and
3 a plurality of analyte detecting members on said substrate;
4 said analyte detecting members arranged to form a plurality of concentric
5 rings on said substrate.

1 24. The device of claim 1 wherein said analyte detecting members
2 includes at least a working electrode and a reference electrode.

1 25. The device of claim 1 wherein said analyte detecting members
2 includes at least a working electrode and a reference electrode and a hydrogel membrane.

1 26. The device of claim 1 wherein said substrate includes at least 100
2 analyte detecting members.

1 27. The device of claim 1 wherein said substrate includes at least 75
2 analyte detecting members.

1 28. The device of claim 1 wherein said substrate includes at least 50
2 analyte detecting members.

1 29. The device of claim 1 further comprising a cartridge containing a
2 plurality of penetrating members, said cartridge coupled to said substrate.

1 30. The device of claim 1 further comprising a cartridge containing a
2 plurality of penetrating members, said cartridge coupled to said substrate so that the
3 analyte detecting members are positioned along an outside rim of the cartridge

1 31. A device for analyte measurement comprising:
2 a substrate; and
3 a plurality of analyte detecting members on said substrate;
4 said analyte detecting members arranged to form a geometric pattern on
5 said substrate.

1 32. The device of claim 31 wherein said substrate includes at least 50
2 analyte detecting members.

1 33. The device of claim 31 wherein said geometric pattern is selected
2 from one of: lines, linear arrays, circular arrays, square arrays, polygonal arrays, or
3 triangular arrays.

1 34. The device of claim 31 further comprising a cartridge containing a
2 plurality of penetrating members, said cartridge coupled to said substrate so that the
3 analyte detecting members are positioned along an outside rim of the cartridge.

1 35. The device of claim 31 further comprising a sample capture
2 assembly that includes a film mesh at the front end to draw blood to the analyte detecting
3 members, and the film can be advanced after use to ensure fresh piece for each lancing
4 event.

1 36. An analyte detecting member comprising: [2736]
2 a substrate;
3 a plurality of conductive lines on said substrate;
4 an insulating layer on said substrate;
5 at least one working electrode and at least one counter electrode, each
6 coupled to at least one conductive line;
7 a cover film;
8 a support layer;
9 a PSA layer

10 wherein there is a hole in cover film
11 wherein there is a hole in PVC support
12 wherein there is a hole in PSA layer;
13 wherein the detecting member is masked to reduce the volume required for
14 the detecting member is 40 nanoliter.

1 37. The device of claim 1 wherein diameter of the hole in cover film: is
2 1 mm; diameter of the hole in PVC support: 1.6 mm, and diameter of the hole in PSA
3 layer: 2.6 mm.

1 38. The device of claim 1 further comprising a radial cartridge having
2 a plurality of said analyte detecting members.

1 39. The device of claim 1 further comprising a ring having a plurality
2 of said analyte detecting members.

1 40. A kit comprising:
2 a device of claim 1;
3 a lancing device, wherein said device of claim 1 is mounted on the lancing
4 device;
5 instructions for use instructing the user to apply no or minimal pressure
6 against the device of claim 1 during lancing and applying pressure post lancing for
7 spontaneous blood generation.

1 41. A method of reducing volume used comprising:
2 masking a surface of an 0.4 μL analyte detecting member;
3 lowering the height of the cover to achieve 40 nL sample volume
4 requirements.

1 42. The method of claim 31 further comprising providing a radial
2 cartridge and forming a plurality of analyte detecting members on said cartridge.

1 43. The method of claim 31 further comprising providing a ring and
2 forming a plurality of analyte detecting members on said ring.

1 44. The method of claim 31 further comprising:

2 providing a ring and forming a plurality of analyte detecting members on
3 said ring.
4 coupling said ring to a cartridge containing a plurality of penetrating
5 members.

1 45. A method of manufacturing comprising:
2 drilling of holes into PVC-support;
3 printing of the conductive lines;
4 printing of the insulating layer;
5 printing of reference and counter electrodes;
6 printing of the working electrode;
7 printing of the hydrophilic membrane;
8 printing of the spacer layer;
9 In process-control: measurement of background and saturation current
10 printing of the PSA-layer;
11 applying of mesh (for the mesh structure);
12 applying of the cover film 126_2 having drilled holes; and
13 stamping.

1 46. The method of claim 45 wherein printing of the working electrode
2 uses a composition: 50% mediator / 100% buffer compounds / 50% GOD

1 47. The method of claim 45 wherein printing of the hydrophilic
2 membrane using a composition: PAA/CHAPS

1 48. The method of claim 45 further comprising in process-control:
2 measurement of background and saturation current after printing spacer layer

1 49. The method of claim 45 applying of the cover film having drilled
2 holes.

1 50. The method of claim 45 control of the resistance when printing the
2 conductive lines.

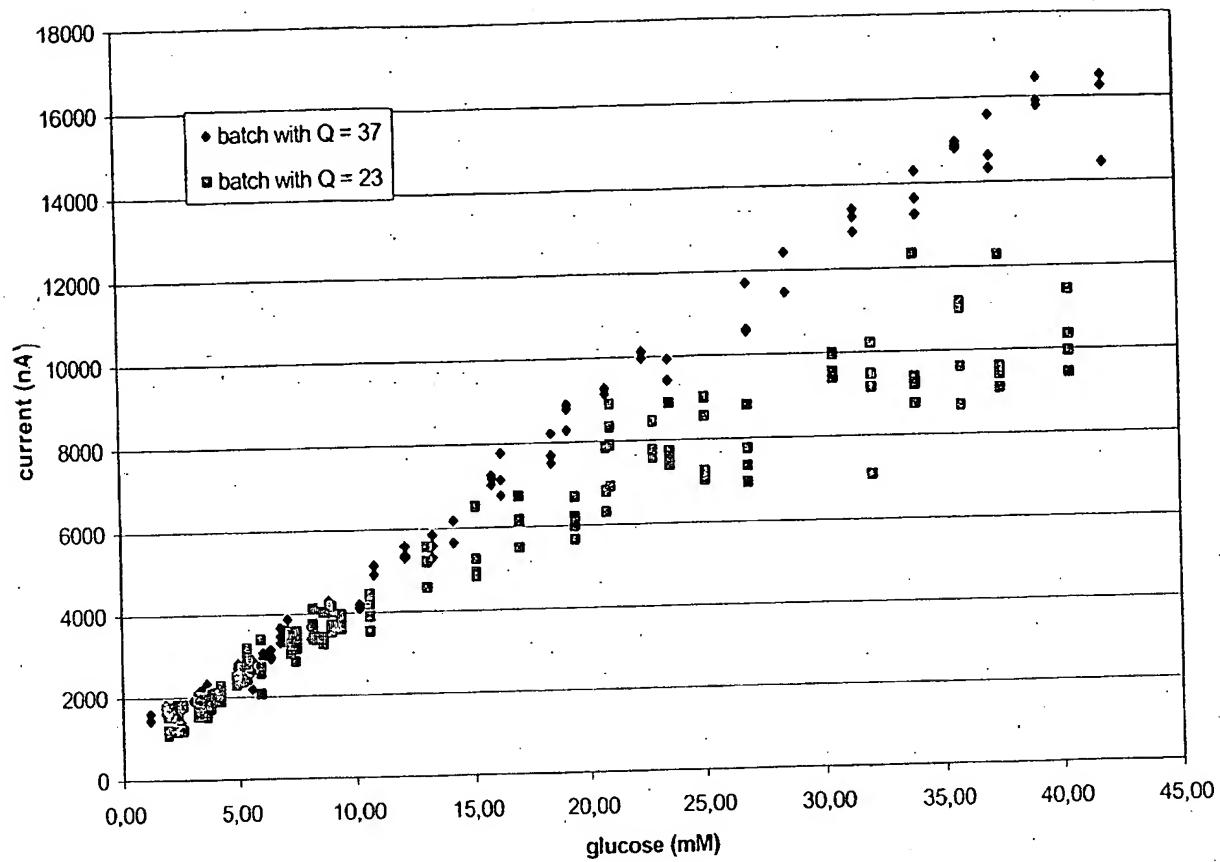


Figure 1

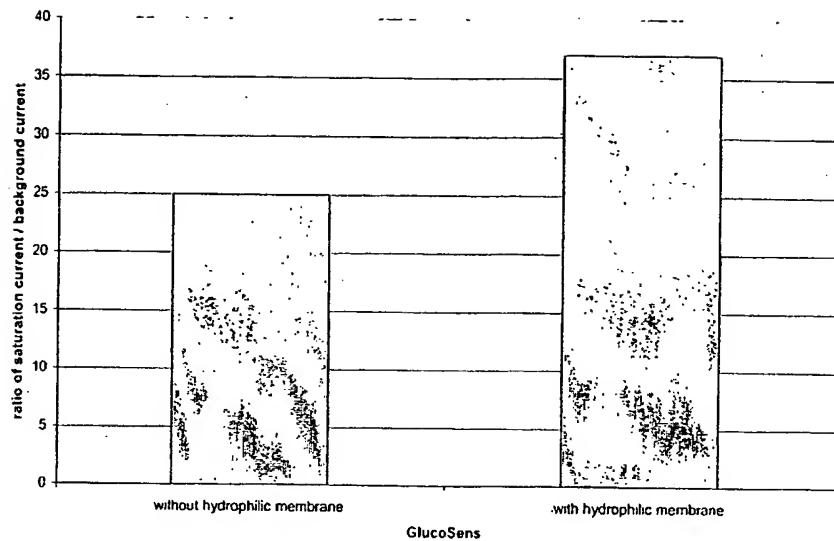


Figure 2

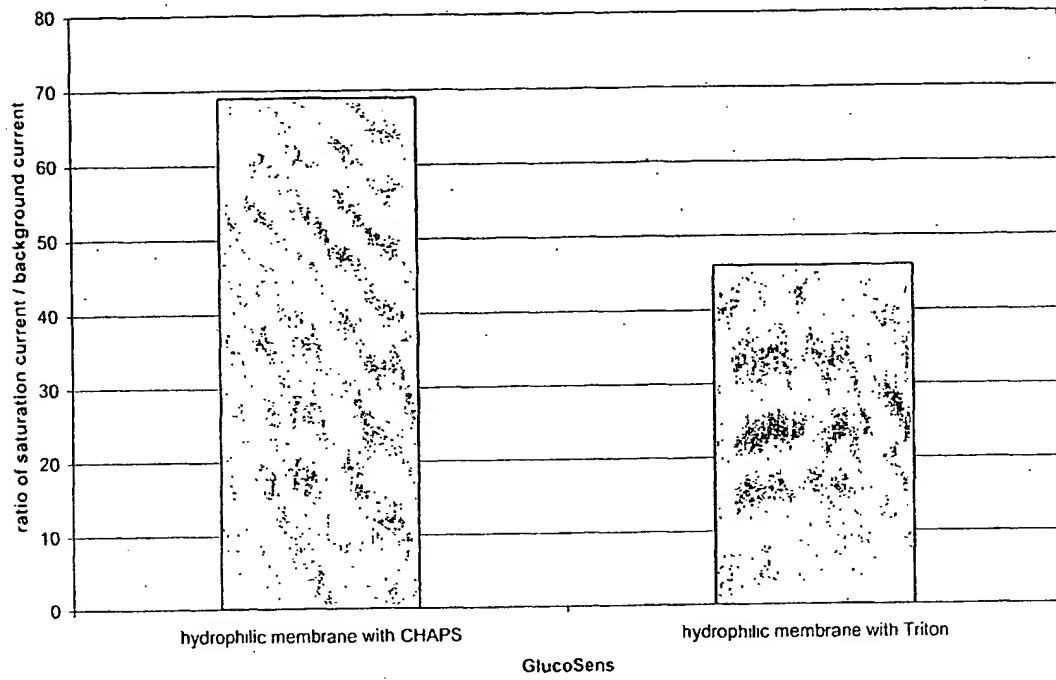


Figure 3

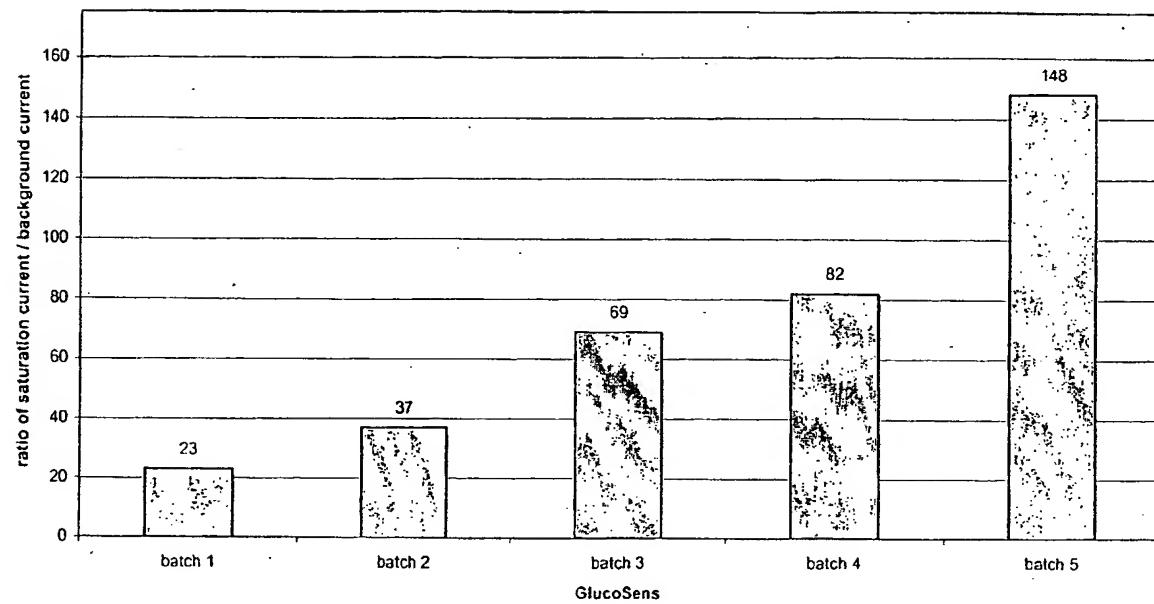


Figure 4

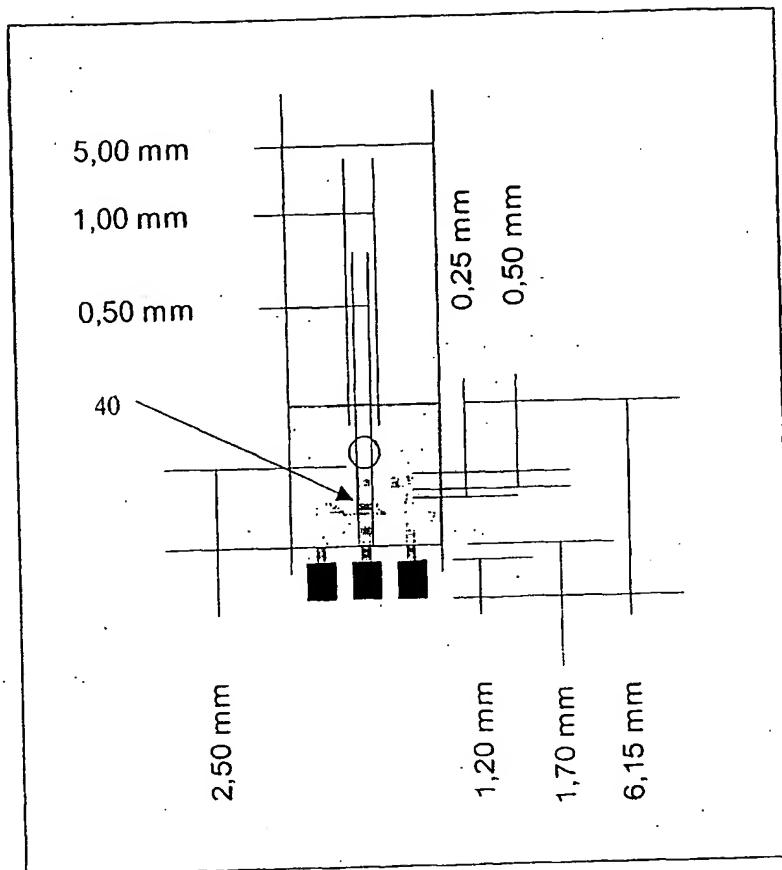


Figure 5A

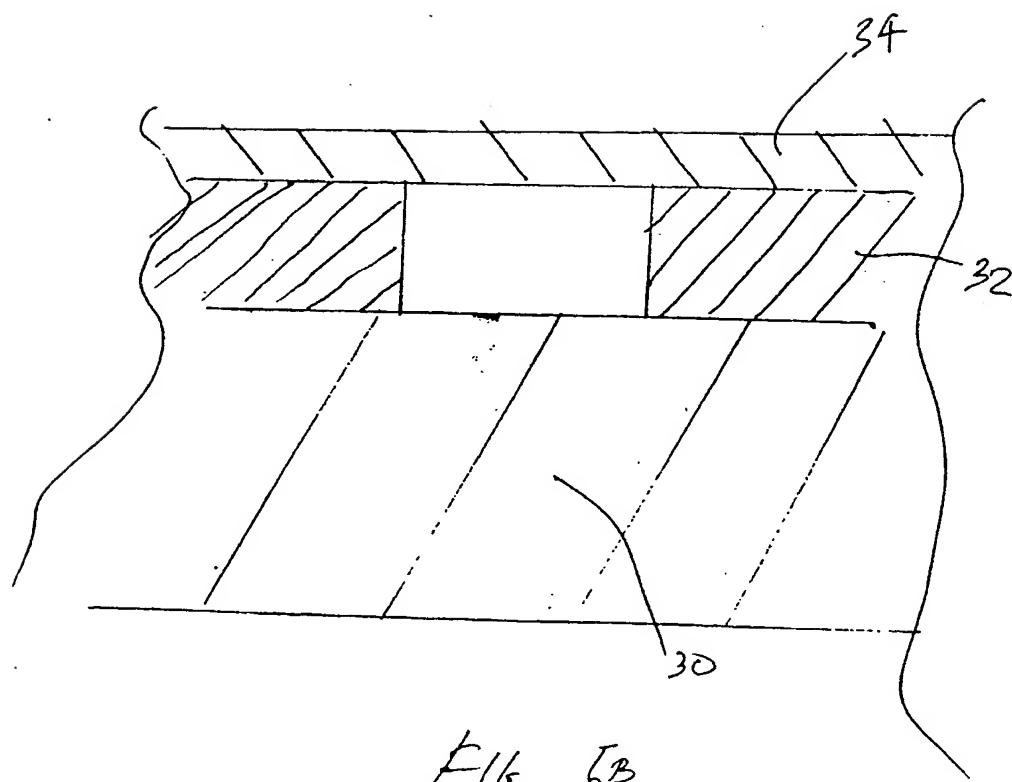
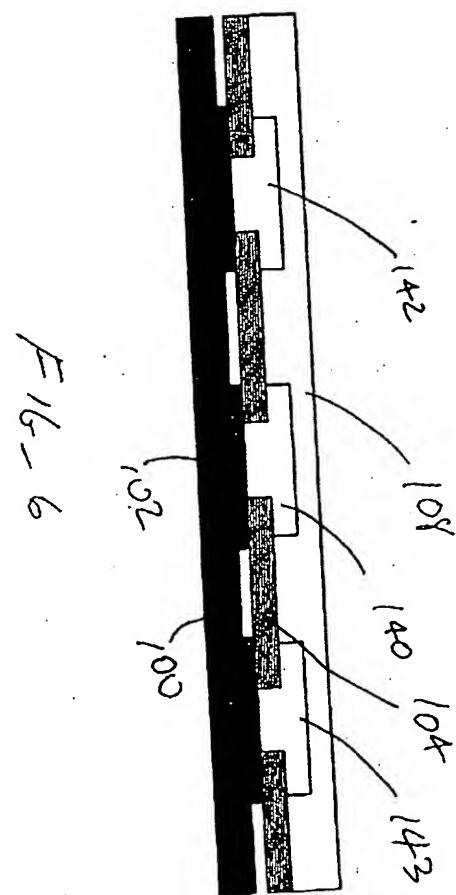


FIG - 5B



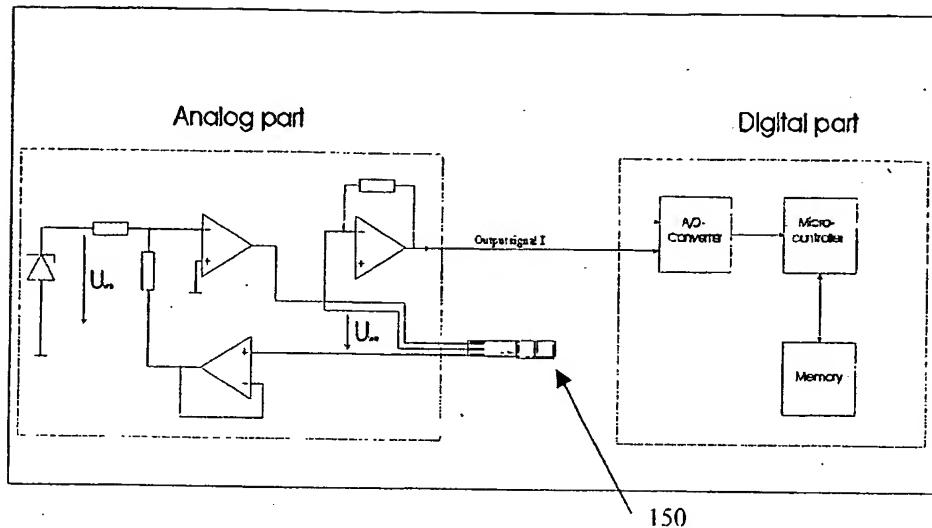


Figure 7A

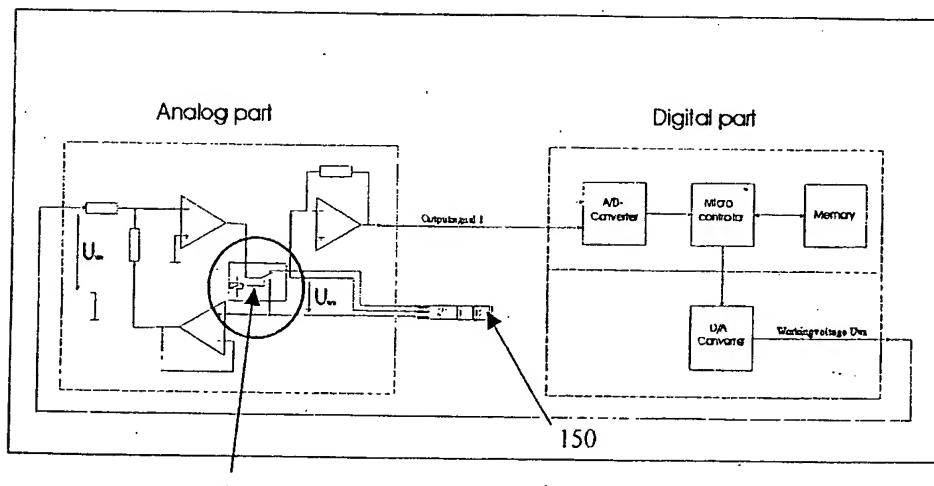


Figure 7B

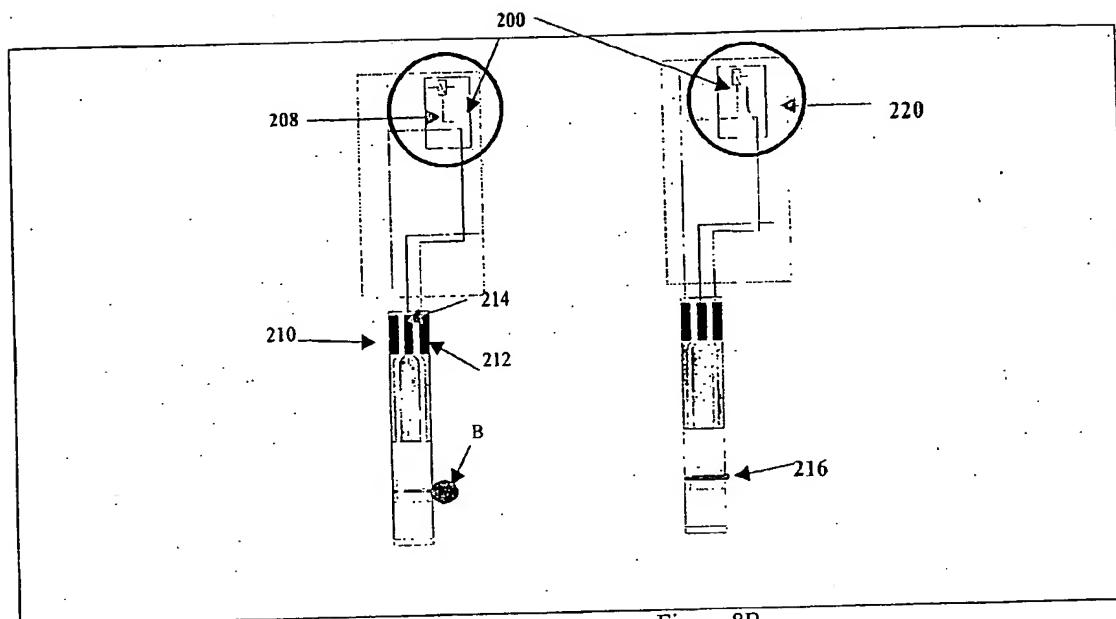


Figure 8A

Figure 8B

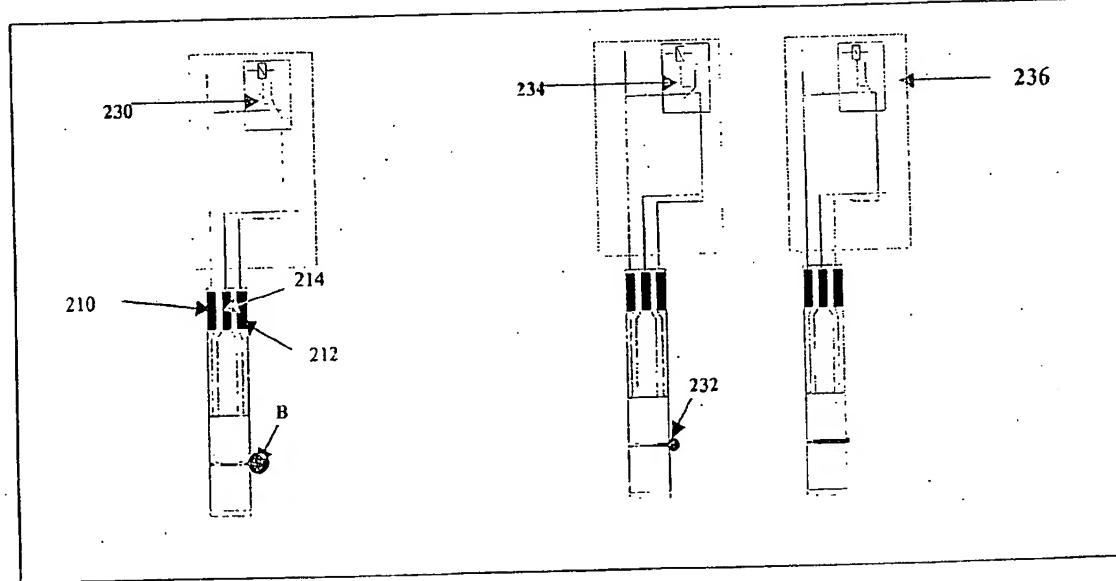


Figure 9A

Figure 9B

Figure 9C

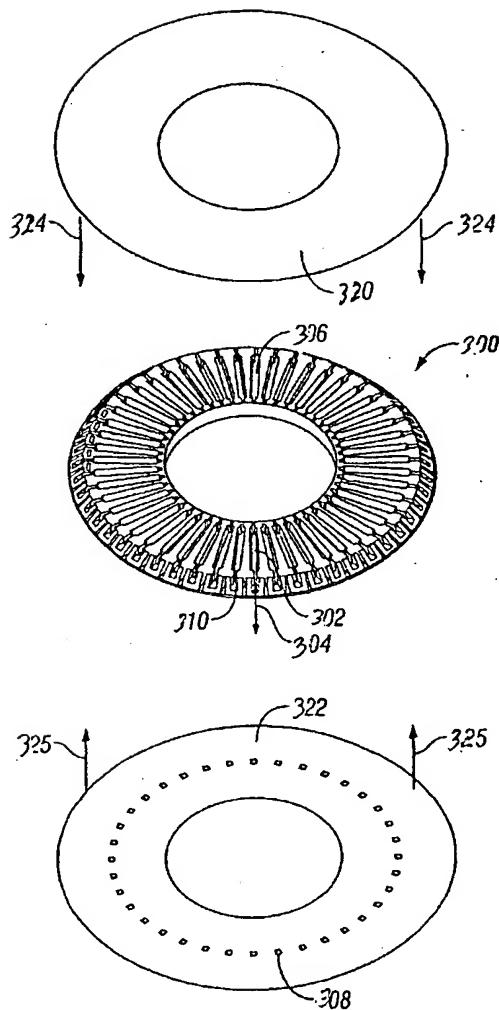


FIG. 10

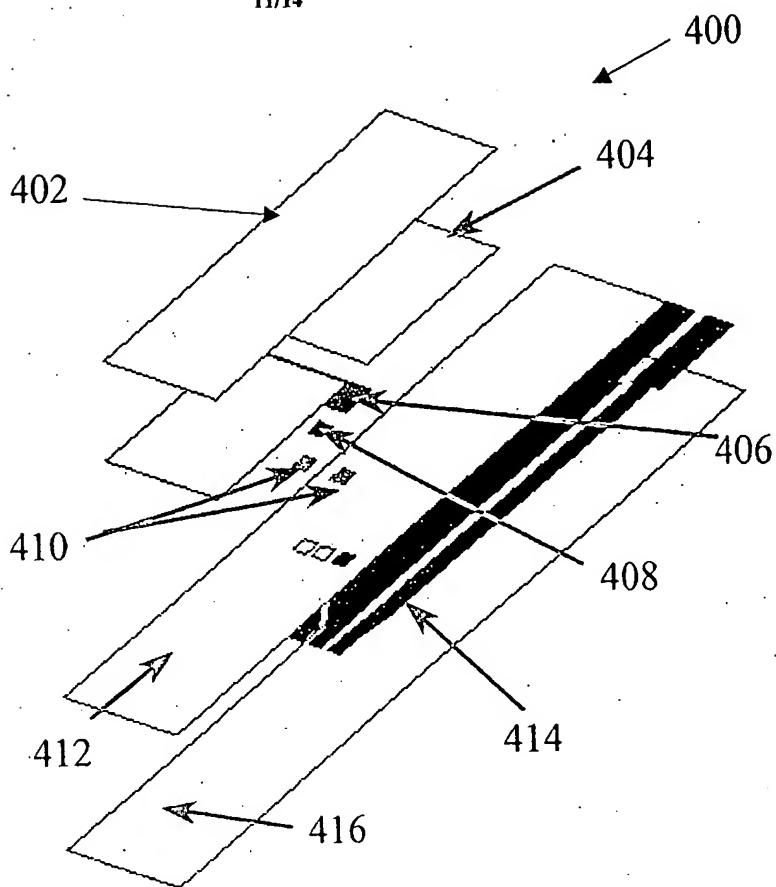


Figure 11

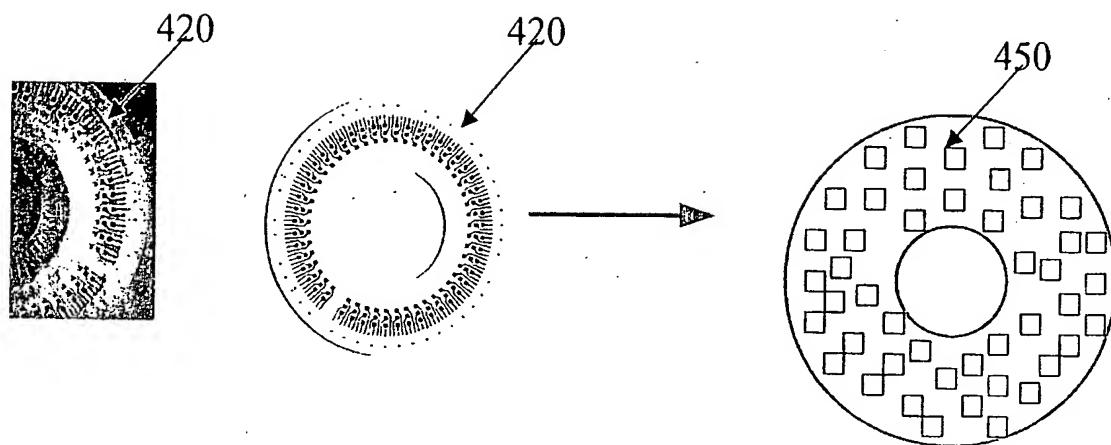


Figure 12

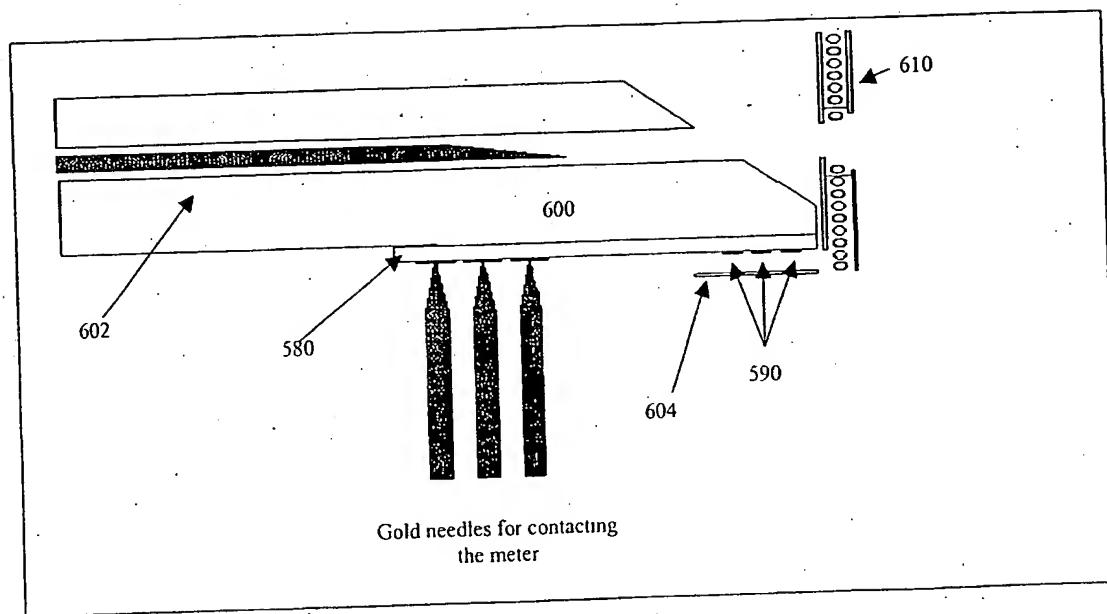


Figure 13

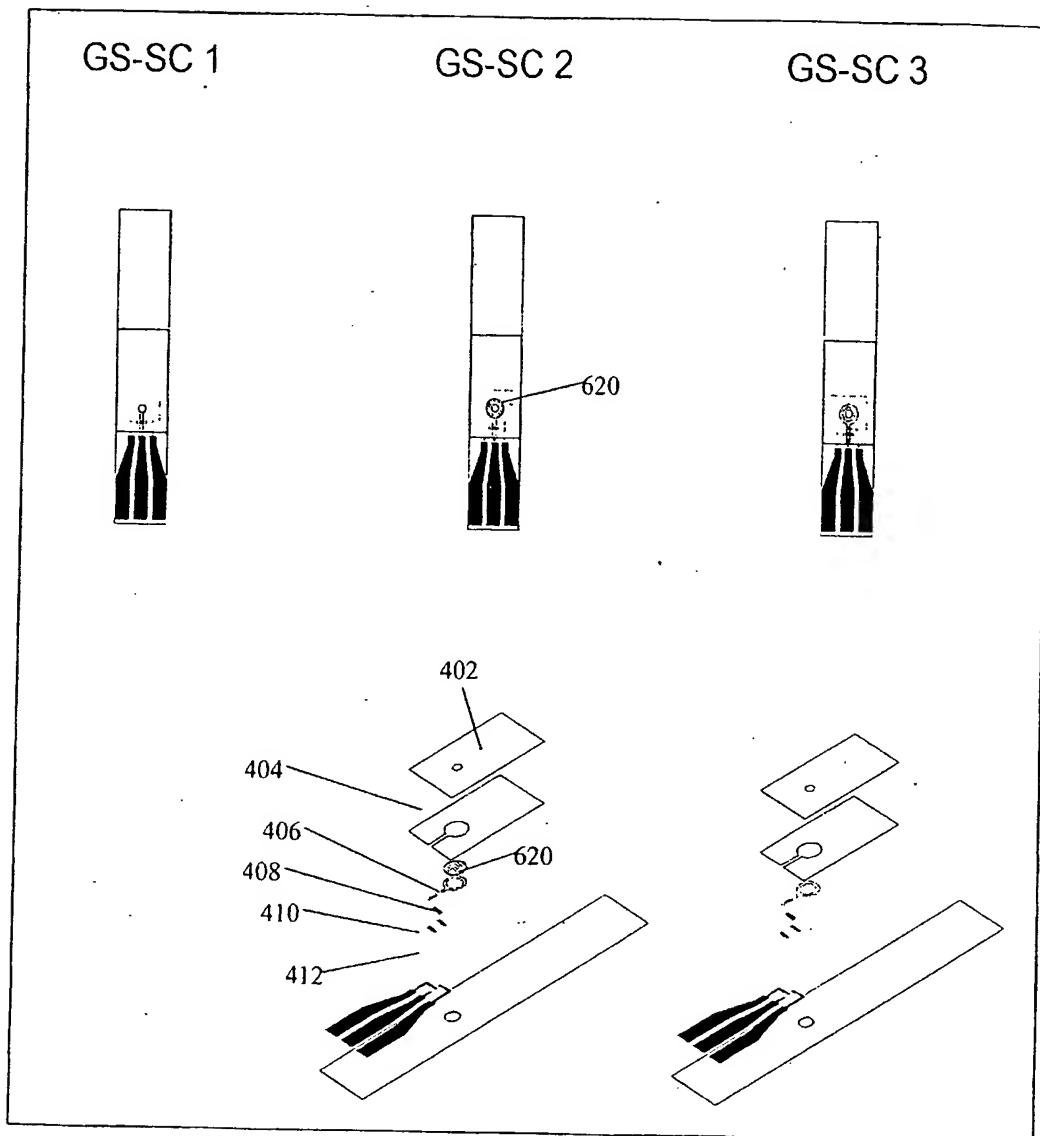


Figure 14

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE LEFT BLANK